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Comparison of Single- and Repeated-Ischemia-Induced Changes in Expression of Flip and Flop Splice Variants of AMPA Receptor Subtypes GluR1 and GluR2 in the Rats Hippocampus CA1 Subregion

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Abstract. In addition to their role in physiological activities, ionotropic glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors (AMPARs) play an important role in neuronal death, especially that following ischemic insults. In this study, we examined the effect of single (SI) and twice repeated (RI)-4-vessel occlusion-ischemia on rat performance in the 8-armed radial maze test. Moreover, the effects of SI and RI on the AMPARs subunits glutamate receptor (GluR) 1 and GluR2 flip and flop variants composition in the CA1 subregion of the hippocampus were investigated using RT-PCR, normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and expressed as their ratios to the latter. The results showed that SI and RI impaired the maze performance by decreasing correct choices and increasing the error choices, but RI increased error choices to a greater extent than the SI. The SI reduced only GluR1 flip/GAPDH on day 1. The SI did not alter ratios of GluR2 variants to those of GluR1. On the other hand, the RI decreased GluR2 flip and flop variants after 1 and 3 days, respectively, whereas after 7 days, it increased the flip variant of both GluR1 and GluR2. Moreover, the RI reduced ratios of GluR2 variants to those of GluR1. These results reveal the differential effects of the SI and RI on memory and expression of the AMPARs subunits GluR1 and GluR2 and their flip and flop variants in the CA1.

Keywords: AMPA GluR1 GluR2 flip and flop, hippocampus CA1, single- and repeated-ischemia, memory, Alzheimer's disease

Introduction

The ionotropic glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors (AMPARs) are homo- or heteromeric complexes of four subunits, termed glutamate receptor (GluR) 1 – 4. The GluR1 and GluR2 are crucial to post-synaptic AMPARs function (1). The ion channel formed by AMPARs subunits, unlike NMDA channels, are Ca^{2+}-permeable and are not blocked by Mg^{2+}, but are highly permeable to another potentially harmful endogenous cation, Zn^{2+} (2).

The AMPARs are important in a variety of physiological processes such as plasticity (3), GluR1 in neuroadaptations that occur following repeated drug administration (4), and GluR2 in learning and food reward (5). On the other hand, compelling evidence supports contributions of AMPARs overactivation (excitotoxicity) to neurodegeneration due to increased Ca^{2+} permeability (2) and activation (not expression) of the c-Jun signaling pathway in ischemic brain damage (6).

The AMPARs are subjected to edition and splicing.

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The second transmembrane domain of the edited GluR2 contains a positively charged arginine, whereas the unedited form contains neutral glutamine (7). The edited GluR2 subunit makes up more than 99% of the GluR2 subunits in rat brain at all developmental stages (7, 8), indicating that the majority of the AMPARs in the adult CNS are Ca^{2+}-impermeable, since GluR2 renders AMPAR impermeable to Ca^{2+} (8). Additionally, each AMPAR subunit exists in two isoforms generated by alternative mRNA splicing of a 115-bp region immediately preceding the fourth transmembrane domain, the so-called 'flip' and 'flop' variants that differentially affect the kinetics of the receptor (9, 10).

The expression of GluR variants are differently changed depending on the regions being investigated. In the hippocampus (HC), GluR2 is the most detected subunit in the CA1 pyramidal cells and type I non-pyramidal cells, whereas GluR1 prevails in the type II non-pyramidal neurons (11). Moreover, a variety of insults could induce different changes in GluR. In the brain, it has been reported that kindling enhances the expression level of the flip variant, but not the flop variants, of both GluR1 and GluR2 mRNAs in the dentate granule neurons (12), whereas GluR1 flip mRNA is slightly decreased by chronic restraint stress in the CA1 (13). Moreover, GluR1 flip, but not GluR1 flop, and both GluR2 flip and flop are upregulated in the rostral ventromedial medulla after induction of inflammatory hyperalgesia (14). In the spinal cord, it has been reported that flip and flop variants are upregulated early in inflammatory phase (15, 16), whereas transcript mRNAs coding for the flop variant had been reported to be decreased in the ventral horn of spinal cord from patients with amyotrophic lateral sclerosis (17).

It has been reported that cerebral ischemia enhances expressions of c-fos and c-jun mRNA (18) and dephosphorylates subfamilies of life-span regulators prior to delayed neuronal death in the vulnerable hippocampal regions (19). In 5-min-ischemia-caused delayed neuronal death in hippocampal CA1 neurons, Akt and calcium /calmodulin-dependent protein kinase kinase-IV activities are decreased after reperfusion, whereas during induction of ischemic tolerance, Akt activity gradually and persistently increases in the CA1 neurons with transient increase in cyclic AMP responsive element-binding protein phosphorylation (20). Moreover, the 4-vessel occlusion ischemia (4-VO) model applied in this study enhances production and release of cytokines and chemokines (21), decreases pyramidal cells in the CA1 (22), and impairs both acquisition and retention in the maze test (23). In our previous study (24), we have determined that single (SI) and twice repeated (RI) 4-VO could differentially induce apoptosis in the CA1 and impair memory. However, there is no comparative study of the effect of the SI and RI on expression of GluR1 and GluR2 splice variants in the CA1. Accordingly, the present study was conducted to investigate the effect of the RI, compared to the SI, on expression of GluR1 and GluR2 flip and flop variants in the CA1.

Materials and Methods

Animals

The experiments were performed on male Wistar rats weighing 230 – 270 g (Kyudo Co., Ltd., Saga). The rats were housed in groups of 5 per cage (30 × 35 × 17 cm) in a room with controlled temperature (23 ± 2°C), relative humidity of 60 ± 2%, and a 12-h light / 12-h dark cycle with light period starting at 7:00 am. Food and water were available ad libitum, except the food during the restricted feeding schedule. The experiments were carried out in compliance with the guidelines stipulated by the Animal Care and Use Committee of Fukuoka University.

Eight-arm radial maze (RAM): apparatus

The RAM apparatus used in this study (Neuroscience Co., Tokyo) consisted of equally spaced transparent Plexiglas eight arms (each 50-cm-long, 10-cm-wide with transparent 50-cm-high side wall) extended from a central octagonal hub (24-cm-across, surrounded by opaque guillotine doors at the entrance of each arm). The maze was elevated 50 cm from the floor. Food cups (3-cm diameter, 1-cm depth, black Plexiglas) were mounted at the end of each arm and served as receptacles for the reinforcers (2 lumps, 50 – 60 mg crystallized sugar) in the baited arms. The experiments were conducted in a room containing many fixed extra-maze visual cues.

Procedures: restricted feeding schedule

The schedule that was applied during this study was achieved by reducing the daily consumption of ration (10 – 12 g/day, CE-2; Clea Japan, Tokyo) so that body weight of each rat was maintained at 80% – 90% of the freely feeding level.

Pretraining, training, and assessment of the RAM performance and drug effects

In pretraining, the animals were acclimatized in groups of 5 rats to the apparatus and the reinforcer food pellets daily (each 10-min session repeated three times at intervals of 60 min) for three days before training. The training phase was started one day after the pretraining and was performed three times/day for 14 days in order to allow the rats to learn how to perform

the RAM task. In the training and drug tests trials, each rat was placed in the central platform, then the guillotine was lifted after 1 min and the rats were allowed to move freely in the apparatus to the baited arms. The trial continued until the test animal had either entered all eight arms and consumed the baits or when 10 min had elapsed. If the test animals proceeded in by using sequential routes consisting of repeating a given angular direction (e.g., 45°) to the neighboring arm, then such animals were excluded from the present experiment. Only the rats that made no errors or only one error for three consecutive days were selected for the study.

Performance assessment

The followings parameters were considered the criteria for the RAM performance: 1: number of correct choices (CC) in the initial 8 chosen arms (entry into an arm that the animal had not previously visited and avoidance of non baited arm) and 2: number of error choices, EC (reentry into an arm that the rat had previously visited and subsequent visit to non-baited arm during the same trial). The CC reflects the extent to which arrangement of area baited during the predelay phase was retained across the delay (reference memory), whereas the EC reflects impaired performance accuracy across successive choices during the post-delay phase, reflecting working memory (25). The RAM performance was observed by a Video Image Motion Analyzer (AXIS 30; Neuroscience Co., Tokyo).

Induction of 4-VO cerebral ischemia

The rats were anesthetized with 50 mg/kg, i.p. sodium pentobarbital and immobilized in a stereotaxic apparatus. The bilateral vertebral arteries were electrocauterized with a bipolar coagulator (MICRO-3D; Mizuho Industrial Co., Tokyo). The bilateral common carotid arteries were then exposed and a hydraulic pressure vascular occluder (OC: 1.5-mm diameter; Technical Supply Co., Osaka) was applied to each exposed artery.

For induction of the SI, the common carotid arteries were bilaterally compressed the next day, with the hydraulic pressure occluders and cerebral circulation was interrupted for 10 min. For the RI, the common carotid arteries were bilaterally occluded for 10 min, and this was repeated once after an interval of 1 h. Body temperature was maintained at 37°C using a heating pad and heating lamp until recovery from anesthesia after surgical operations or the righting reflex reappeared following occlusion of carotid arteries. Rats that did not exhibit loss of righting reflex during arterial occlusion were excluded from subsequent experiments. Rats that only underwent cauterization of the vertebral arteries, then had occluders fitted on the common carotid arteries but without occlusion, were used as sham-operated controls.

Reverse transcription-polymerase chain reaction (RT-PCR) of apoptosis-related gene mRNA

The brain was dissected and put immediately onto dry ice. The dorsal HC containing CA1 was punched out (2-mm diameter) at A = –2.8, L = 1.0, H = 3.2 (26). For total RNA isolation, the tissue was homogenized and mixed with 1000 μL Trizol (Gibco BRL, Grand Island, NY, USA) and 200 μL chloroform, shaken, and centrifuged at 12,000 × g for 10 min at 4°C. The uppermost part of the supernatant was mixed with 500 μL of each of isopropanol and cooled ethanol 75%, shaken, and centrifuged at 7,500 × g for 15 min at 4°C. The supernatant was discarded, and the pellet was diluted with 20 μL deionized water. The RNA was quantified by measuring the absorbency of the sample at 260 nm.

Reverse transcription into first strand complementary DNA (cDNA) was conducted by mixing 5 μg of total RNA and 1 μL oligo one (dT) 12-18 primer and Superscript II reverse transcriptase (Gibco BRL) and incubating at 70°C (ASTEC Block Incubator; ASTEC, Tokyo). The reverse-transcribed cDNAs were subjected to PCR in PCR reaction mixture to assess the expression of GluR1 and GluR2 flip and flop variants and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNA using a PCR reagent system (Gibco BRL, Gaithersburg, MD, USA), containing 2 μL 10 × PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 1 μL 50 mM $MgCl_2$, 1 μL 10 mM dNTP mix, 1 μL 10 μM amplification sense or antisense primer 2×, 0.5 μL Taq DNA (2.5 units/0.5 μL) polymerase, 1 μL cDNA, and 40 μL autoclaved de-ionized water. The mixture was subjected to first denaturation at 94°C for 2 min, and then the medium was subjected to 35 cycles each of 1-min denaturation at 94°C, annealing at 55°C, and extension at 72°C using a programmed temperature control system (PC-701, ASTEC). For quantification of the PCR products, 9 μL of the product was mixed with 1 μL loading buffer (1% SDS, 50% glycerol, 0.05% bromophenol blue; Takara, Shiga) and electrophorized together with the ladder (Ready-Load 100-bp DNA Ladder; Invitrogen, Carlsbad, CA, USA) on 1% agarose for 20 min at 150 V (Mupid, Advance Co., Ltd., Tokyo). The agarose gel was prepared with TBE buffer (Gibco BRL). The gel was scanned with ultraviolet irradiation after staining with ethidium bromide (1 μg/ml) for 20 min. Finally, the densities of the bands on the agarose gels were measured using the NIH Image Program (Version 1.6.2). The sequences of the primers were as

follows: GluR1: F-5'-AGA GGG ACG AGA CCA GAC AAC CAG-3' (1713 – 1736), R-flip: 5'-GCT GGT CTT GTC CTT ACT TCC GGA-3' (2374 – 2397), R-flop: 5'-GCT GGT CTT GTC CTT GGA GTC ACC-3' (2374 – 2397) (GenBank accession No. M36418); GluR2: F-5'-AGA TGG AAG AGA AAC ACA AAG TAG-3' (2049 – 2072), R-flip: 5'-ACT GGT CTT TTC CTT ACT TCC CGA-3' (2710 – 2733), R-flop: 5'-ACT GGT CTT TTC CTT GGA ATC ACC-3' (2710 – 2733) (GenBank accession No. M85035); GAPDH: F-5'-TGA AGG TCG GTG TCA ACG GAT TTG GC-3' (35 – 60), R-5'-CAT GTA GGC CAT GAG GTC CAC CAC-3' (994 – 1017) (GenBank accession No. M17701).

To ensure that the amount of template used in the PCR reaction was in the linear range and PCR reaction did not reach saturation, we performed a preliminary analysis to determine the relationship of PCR product accumulation as a function of both the number of cycles and the amount of RNAs used in the RT reaction. Mock RT-PCR reaction controls were performed by omitting primers, using templates derived from RT reactions lacking either reverse transcriptase or total RNAs. No specific PCR product was found in control reactions.

Statistical analyses

The effect of the SI and RI were evaluated using one-way ANOVA, and the differences between the SI and RI were evaluated by two-way ANOVA using 2 (groups) × 4 factorial table (four measurements for control, days 1, 3, and 7). Post-hoc comparisons were made by Tukey's test. The ratio values were analyzed by the *t*-test. Data are presented as means ± S.E.M. $P<0.05$ was considered statistically significant in all tests.

Results

Effects on the RAM performance

The performance of control rats was characterized by 7.5 ± 0.16 CC and 0.5 ± 0.16 EC. Figure 1 shows that both the SI and RI impaired the RAM performance by decreasing the CC ($F_{2,25} = 13.2$, $P<0.01$) and increasing the EC ($F_{2,25} = 20.2$, $P<0.001$). The SI decreased CC to 6.5 ± 0.22 ($P<0.01$) but increased the EC to 5.17 ± 1.19 ($P<0.05$). On the other hand, the RI decreased the CC to 5.9 ± 0.26 ($P<0.001$) but increased EC to 10.1 ± 1.4 ($P<0.001$). Analysis of data also showed that the RI increased the EC to a greater extent than the SI ($P<0.025$). No significant difference was detected between the SI and RI effects on the CC.

The SI-induced time-dependent changes of GluR1 and GluR2 flip and flop variants

We next examined whether there were changes in expression of GluR1 and GluR2 mRNAs in the CA1 after ischemia. The SI decreased GluR1 flip ($F_{3,23} = 4.4$, $P<0.02$). When the variants expression levels were normalized with regard to GAPDH, it was found that the SI decreased the ratios of GluR1 and GluR2 slip and flop variants to GAPDH on day 1, but only the ratio GluR1 flip/GAPDH reached a significant ($P<0.025$) level (Fig. 2A). Moreover, it could be seen from Fig. 2B that on day 1, the SI non-significantly decreased the GluR2 flip and flop/GAPDH ratio. No changes in GluR1 or GluR2 flip and flop variants were induced by the SI on days 3 and 7.

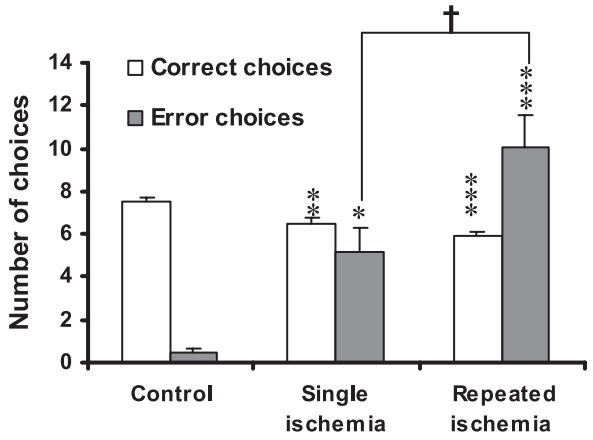

Fig. 1. Effects of single and repeated ischemia on the eight-arm radial maze performance assessed as the numbers of correct or error choices. Data are means ± S.E.M. Statistically significant differences: *$P<0.05$, **$P<0.01$, ***$P<0.001$ *vs* sham; †$P<0.025$, repeated *vs* single ischemia.

The RI-induced time-dependent changes of GluR1 and GluR2 flip and flop variants

The RI changed expression of GluR1 flip ($F_{3,63} = 4.8$, $P<0.005$) and flop ($F_{3,63} = 5.3$, $P<0.002$). On the other hand, the RI changed GluR2 flip ($F_{3,63} = 4.7$, $P<0.005$) and GluR2 flop ($F_{3,63} = 2.8$, $P<0.05$). When the variants expression levels were normalized with regard to GAPDH, it was found that the RI did not significantly change GluR1 flip and flop/GAPDH ratios on days 1 – 3. However, on day 7, the RI significantly ($P<0.05$) increased GluR1 flip/GAPDH ratio (Fig. 3A). On the other hand, the RI significantly ($P<0.01$) decreased GluR2 flip/GAPDH on day 1, whereas it decreased ($P<0.05$) GluR2 flop/GAPDH ratio on day 3. Moreover, the RI increased ($P<0.05$) GluR2 flip /GAPDH on day 7 (Fig. 3B).

Analysis of changes in ratios of GluR2 variants to those of GluR1 showed that the SI did not significantly change any ratio (Fig. 4A). On the other hand, it could be seen from Fig. 4B that the RI decreased the ratio of

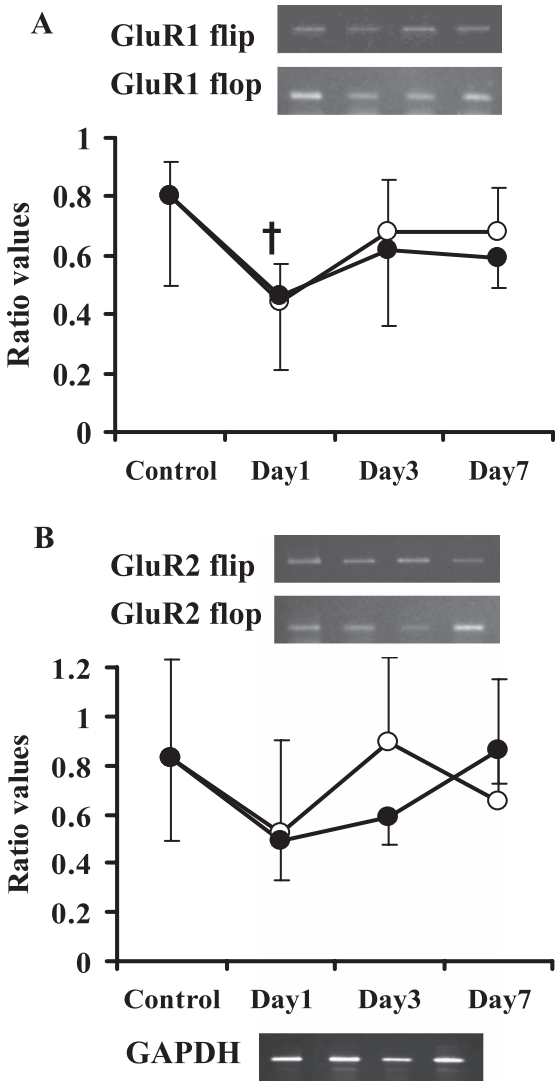

Fig. 2. Effect of single ischemia on GluR1 (A) and GluR2 (B) flip (open circle) and flop (closed circle) variants mRNA levels in the CA1 subregion of hippocampus. The bands are representative agarose gel electrophoresis patterns obtained by polymerase chain reaction of the GluR variants and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bands from left to right within each representation are for control and ischemic rats 1, 3, and 7 days after ischemia. The mean ± S.E.M. of the ratio of each variant to GAPDH is reported. Statistically significant differences: $^{\dagger}P<0.025$, *vs* control.

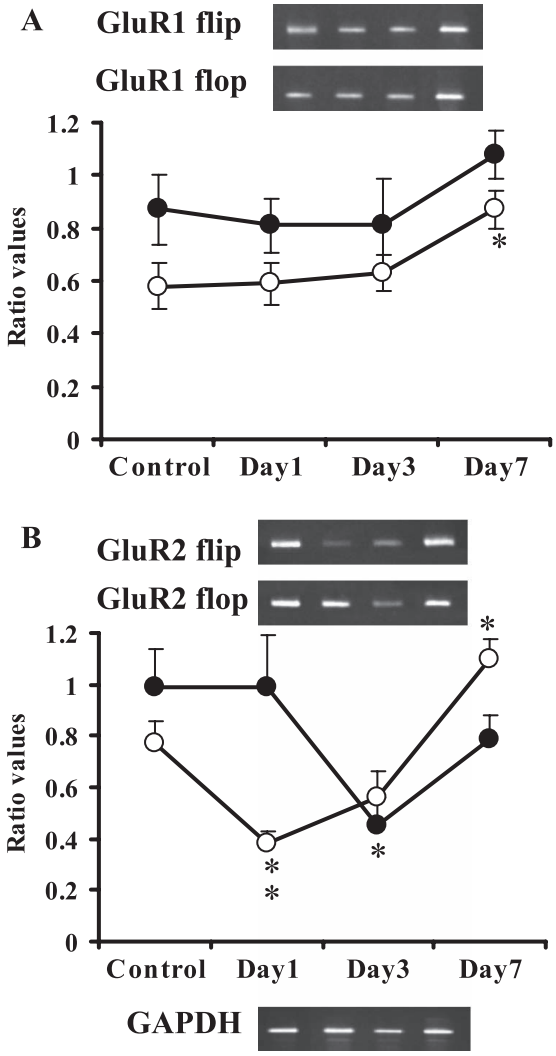

Fig. 3. Effect of repeated ischemia on GluR1 (A) and GluR2 (B) flip (open circle) and flop (closed circle) variants mRNA levels in the CA1 subregion of hippocampus. The bands are representative agarose gel electrophoresis patterns obtained by polymerase chain reaction of the GluR variants and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The bands from left to right within each representation are for control and ischemic rats 1, 3, and 7 days after ischemia. The mean ± S.E.M. of the ratio of each variant to GAPDH is reported. Statistically significant differences: $*P<0.05$, $**P<0.01$, *vs* control.

GluR2 flip/GluR1 flip on days 1, 3, and 7 ($P<0.05$). Moreover, the RI decreased GluR2 flip/GluR1 flop only on day 1 ($P<0.05$). On the other hand, the RI decreased GluR2 flop/GluR1 flip on day 3 ($P<0.01$) and day 7 ($P<0.001$). The RI also decreased GluR2 flop/GluR1 flop on days 3 and 7 ($P<0.01$).

Comparison of the SI- and RI-induced changes revealed a significant difference between the two ischemia models regarding their effects on GluR1 flip ($F1,87 = 87.72$; $P<0.0001$ for the main effect of ischemia type; $F3,87 = 5.9$, $P<0.001$ for differences among the days; with no significant group × time interaction $F3,87 = 32.36$, $P<0.077$) and GluR1 flop ($F1,87 = 40.3$, $P<0.0001$ for the main effect of ischemia type; $F3,87 = 4.55$, $P<0.005$ for differences among the days; $F3,87 = 3.24$, $P<0.026$ group × time interaction). On the other hand, no significant difference was detected for effects on GluR2 flip or flop. However, in case of the effect on GluR2 flip, a significant difference was detected for time ($F3,87 = 3.71$, $P<0.015$), suggesting

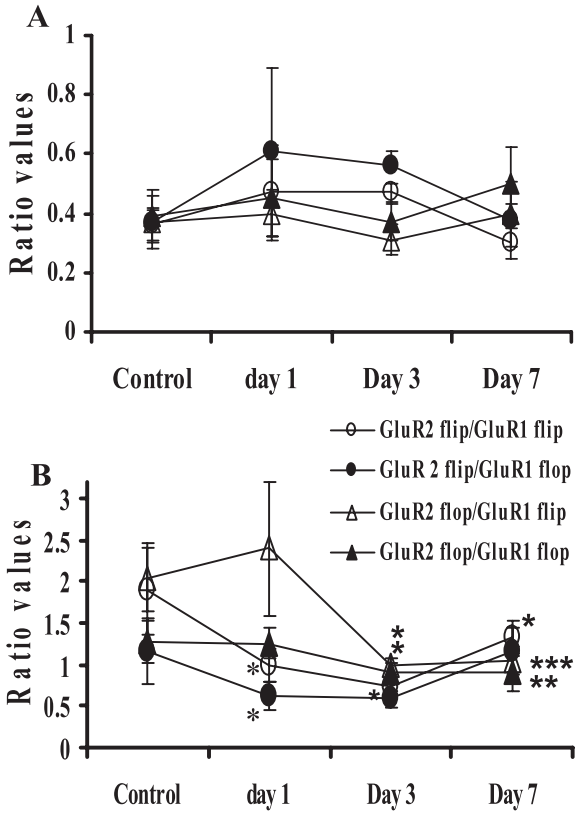

Fig. 4. Changes in the ratios of GluR2 flip and flop to those of GluR1 caused by single ischemia (A) and repeated ischemia (B). Values are each a mean ± S.E.M. Statistically significant differences: **P*<0.05, ***P*<0.01, ****P*<0.001, *vs* control.

different effects by the SI and RI at the time points examined in this study.

Discussion

Ischemia differentially regulates expression of GluR1 and GluR2 in the HC. The intensity of ischemia-damage, the AMPAR variant, and the HC subregion involved depend on the vessel(s) occluded, duration and frequency of occlusion and reperfusion. The present results showed that the SI decreased GluR1 flip expression on day 1, whereas the RI decreased the GluR2 flip and flop variants on days 1 and 3. Our results agree with the reports of significant decrease in total protein synthesis (27, 28), GluR2 protein synthesis (28), and the levels of GluR2 flip and flop variants (29) in the CA1 subregion. However, we observed different effects for the SI and RI. The RI, differently from SI, seems to specifically affect the neurons expressing GluR2 since it did not decrease GluR1 but specifically decreased GluR2 and its ratio to GluR1 variants. The differential changes of GluR2 and GluR1 and/or their variants after the second reperfusion could be due to involvement of GluR1 and to lesser extent GluR2 in the first ischemic insult/reperfusion, whereas GluR1 are spared in the case of the second reperfusion. This difference could be attributed to the properties of GluR1, GluR2, and their variants. Different subunits and splice variants within the same subunit could display different kinetic properties. The GluR2 desensitizes at a slower rate than GluR1, and GluR2 flip displays the slowest desensitization to the AMPAR agonist (30). Different distribution of various subunits on distinct neuronal population could be another factor in the difference in GluR1 and GluR2 change induced by either the SI or RI. The GluR1 prevails not only in the pyramidals but also on GABAergic neurons (31). These neurons are protected from dying due to the presence in these cells of the Ca^{2+}-binding protein parvalbumin (32), which may buffer against the excessive Ca^{2+} influx associated with sustained activation of receptors containing the GluR1 subunit (9). The decreased GluR2 flop mRNA after the RI is similar to that reported in our recent study (33). The reduction of GluR2 could be due to promotion by the ischemic insults of internalization of GluR2-containing AMPARs from synaptic sites via clathrin-dependent endocytosis and facilitation of delivery of GluR2-lacking AMPARs to synaptic sites via soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor-dependent exocytosis, evident after 24 h (34). The decrease of GluR2 mRNA by the ischemic insult triggers degeneration of the CA1 pyramidal cells (35) by mechanisms involving excessive release of glutamate and increased oxygen free-radical formation (36) and Ca^{2+} permeability (37). Moreover, the decrease of GluR2 seems important in the pathological mechanisms underlying stroke, hence the neuroprotective effect produced by treatments like *toki-shakuyaku-san* in the RI model reverses the GluR2 reduction (33).

The ischemia-related reduction of GluR seems to be specific to the CA1 neurons since CA3 neurons are relatively spared (38). This reduction is more specifically observed in the CA1 pyramidal neurons (39) that could regulate the AMPAR subunit expression through changes in protein synthesis and stability (28). Although both GluR1 and GluR2 are expressed in the CA1 subregion (40), it could be suggested that reduction of GluR2 is important in the hippocampal neuronal death and the subsequent behavioral disorders. It has been reported that the CA1 neurons destined to die after ischemia exhibit reduced levels of GluR2 protein expression immediately before cell death (41). The reduced GluR2 level could be restored by fimbria-fornix deafferentiation, which interrupts the afferent input to the CA1 and protects the CA1 neurons at 7 days post-

ischemia (42).

The ischemia-induced neuronal death is also time-dependent. It has been reported that short anoxia /hypoglycemia increases GluR1 and GluR2/3 subunits 1 h, but not 15 min, after the episode (43). Moreover, occlusion of the common carotid artery alone for 5 min decreases GluR2, but not the GluR1, in the CA1 (41). In this study, reduction of GluR1 was observed on day 1 after the SI, whereas reduction of GluR2 variants was observed 1 – 3 days after the RI. The temporal effect of ischemia could be due to the time required for total protein and GluR2 protein synthesis to decrease in the CA1 (28) or for hyperactivation of astrocytes (39), GFAP expression in reactive astrocytes (44), and intensification of the microglial reaction (45) that all decrease GluR2 expression. It could be suggested that the SI of 10 min is of an intensity adequate to induce changes in GluR1 on day 1. On the other hand, in the RI, the second episode following a reperfusion induces delayed change of GluR2. This delay coincides with delayed neuronal death in the CA1 subregion of the HC reported 3 days after transient forebrain ischemia (38).

The results also showed that the RI significantly increased especially the flip variant of GluR on day 7. The upregulation of GluRs gene could be attributed to mechanisms such as polyadenylation of the existing GluR pool, possibly on surviving pyramidals that do not die in ischemia (46), or increase in the rate of turnover of GluR subunit (16). The increased GluR1 flip variant on day 7 could be involved in greater neuronal degeneration and consequent higher error choices in the RI by two mechanisms. Firstly, in keeping with the properties of the flip variant of GluR, it could be suggested that an increase in the GluR1 flip subunit expression may reduce the desensitization rate of the AMPARs (30), induce stronger channel opening activity, and increase Ca^{2+} permeability, leading to long-lasting enhanced efficiency of fast synaptic transmission in vulnerable pyramidal neurons of the CA1 subregion in the HC (29). These changes eventually increase AMPAR-mediated currents, rendering the excitatory neurons more susceptible to excitatory amino acid (glutamate)-induced excitotoxic damage (47). Secondly, increase of GluR1 leads to decreased GluR2/GluR1 ratio. Taken together, it is plausible to suggest that increased GluR1 expression, mainly the flip variant, could be involved in the greater memory impairment caused by the RI in this study, possibly due to more neuronal death reported 7 days after reperfusion in the RI (48). However, since the SI also impaired memory, the early decrease in GluR on day 1 also participates in memory impairment, albeit increased by the changes produced 3 – 7 days later. It should be noted that our results showed that there was also a trend to increase GluR2 flip in the RI. This upregulation could indicate a possible mechanism for enhanced AMPAR desensitization leading to development of tolerance to ischemia (49). However, the increase of GluR2 did not parallel the increased GluR1, indicating a greater neurodegenerative process than the adaptive/protective ones in the RI. Moreover, although slightly upregulated, these GluR2 are not impermeable to Ca^{2+} due to the possible disruption of Q/R site edition in the CA1 hippocampal pyramidal neurons by ischemia, an end result contributing to the delayed selective degeneration of the CA1 hippocampal pyramidal neurons (50).

In conclusion, the RI caused greater error choices than the SI. The SI decreased GluR variants, especially GluR1 flip, after 1 day. The RI decreased GluR2 flip and flop variants after 1 and 3 days, respectively, whereas after 7 days, it decreased GluR2:GluR1 ratio, and increased the flip variant of both GluR1 and GluR2. Treatments directed to increase GluR2 or decrease GluR1, especially the flip variants, could be one of the conceivable strategic drug therapies for prevention or improvement of memory deficit in the acute or chronic stage of cerebral ischemia.

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